



Multi-center analytical evaluation of a novel automated tacrolimus immunoassay[☆]



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ABSTRACT

Background: Tacrolimus (TAC) is a post-transplantation immunosuppressant drug used in patients for whom careful monitoring of TAC concentration is essential. A new semi-automated immunoassay for TAC measurement, the Elecsys Tacrolimus assay, is available and has been assessed in a multi-center evaluation.

Methods: Residual whole blood samples from patients undergoing TAC therapy after organ transplant were used in assay evaluation at five clinical laboratories in Europe. Experiments included imprecision according to CLSI EP5-A2 (within-run and intermediate), functional sensitivity, linearity according to CLSI EP6-A, and recovery from external quality assessment scheme (EQAS) samples. The assay was compared to LC–MS/MS used routinely at each investigational site, and to the Abbott Architect immunoassay.

Results: Linearity from 0.5 to 40 µg/L was observed and functional sensitivity of 0.3 µg/L (CV ≤ 20%) was determined. Within-run imprecision was ≤ 5.1% on cobas e 602 (5.1% at 1.5 µg/L) and ≤ 8.9% (8.9% at 0.8 µg/L) on cobas e 411. The intermediate imprecision for TAC concentrations ≥ 6.8 µg/L was ≤ 6.5%. At lower therapeutic concentrations (to 1.5 µg/L) it was consistently ≤ 10%. Deming regression analysis of method comparison to LC–MS/MS yielded slopes of 1.07 (95%CI: 1.05/1.10) for heart transplant samples, 1.13 (95%CI: 1.09/1.16) for kidney, and 1.05 (95%CI: 1.02/1.08) for lung transplant samples.

Conclusions: The Elecsys Tacrolimus assay has good linearity, functional sensitivity and intermediate imprecision and is comparable to LC–MS/MS methods. The over-all performance of ECLIA demonstrates a modern generation TAC assay that meets the demands of monitoring drug concentrations in current immuno-suppressive regimens.

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Abbreviations: TAC, tacrolimus; TDM, therapeutic drug monitoring; OPTN/SRTR, Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients; LOQ, limit of quantification; LC–MS/MS, liquid chromatography tandem mass spectrometry; IPT, International Proficiency Testing Scheme; CMIA, chemiluminescent microparticle immunoassay; MCE, multi-center evaluation; ECLIA, electrochemiluminescence immunoassay; QC, quality control; EQAS, external quality assessment scheme; HTx, heart transplant; KTx, kidney transplant; LTx, liver transplant; IRB/EC, Internal Review Board or Ethics Committee; GCP, good clinical practice; CV, coefficient of variation; ACMA, antibody-conjugated magnetic immunoassay.

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Introduction

The discovery of the second generation calcineurin inhibitor tacrolimus (TAC) in the early 1990s has greatly enhanced the therapeutic success of organ transplantation initiated by cyclosporine A as immunosuppressive therapy [1]. Therapeutic drug monitoring (TDM) was recommended with the introduction of TAC, and undoubtedly contributed to this success. According to the 2011 OPTN/SRTR annual report [2], over 80% of solid organ recipients received TAC. However, the calcineurin inhibitor toxicity profile remains a major concern and may affect long-term outcome for many patients, which is today the main challenge for transplantation medicine [1,3].

New strategies to improve the long-term preservation of organ function and to reduce the incidence of accompanying diseases (e.g. infections, renal insufficiency, cardiovascular disease and malignancy)

Table 1
Comparison of LC–MS/MS measurement methods used by investigational sites in the tacrolimus MCE.

Site	Extraction ^{a,d}	Calibrators	LC/MS manufacturer/model ^{b,c}	Analytical column	Method working range	Within-lab imprecision
s-1 [32,33]	PPT	Chromsystems 6PLUS1	Waters Alliance 2695	MZ-Analysentechnik MZ Aqua Perfect C18	0.5–50 µg/L	<7%
s-2	PPT + on-line SPE	Chromsystems 6PLUS1	HPLC/Quattro micro API	150 × 3.0 mm, 5 µm	2.1–42.4 µg/L	<5%
s-3	PPT	RECIPE ClinCal	Waters Alliance 2795	Waters Sunfire C18 2.1 × 100 mm,	1.0–46 µg/L	<4%
s-4	PPT	Chromsystems MassCheck	HPLC/Quattro Ultima Pt	5 µm	0.5–30.3 µg/L	<8%
s-5	PPT	RECIPE ClinCal	Agilent Infinity 1290	Agilent Zorbax Eclipse XDB C18 4.6 × 50 mm,	0.6–44.7 µg/L	<6%
			HPLC/Agilent 6460	1.8 µm		
			Waters ACQUITY UPLC/TQD	Waters MassTrakTM TDM C18 2.1 × 10 mm		
			Waters ACQUITY UPLC/TQD	Waters MassTrakTM TDM C18 2.1 × 10 mm		

^a All LC–MS/MS laboratories used ascomycin as an internal standard except site Barcelona [13Cd2-tacrolimus].

^b All laboratories used step gradient with mobile phases consisting of ammonium acetate and formic acid in water or methanol for LC except for Munich [methanol/2 mM ammonium acetate].

^c All laboratories used electrospray ionization in the positive mode (ESI+) for mass spectrometry.

^d PPT, precipitation with organic solvent mixture and centrifugation; SPE, solid phase extraction.

associated with immunosuppression are increasingly important. One strategy is combining TAC with drugs displaying a different mode of action, minimizing dose requirement and related side effects [4–6]. Target TAC concentrations are now 5–10 µg/L (heart and liver) and 3–7 µg/L (kidney) for stable transplant recipients with current therapeutic protocols [7]. Dose minimization means concentration minimization, creating new challenges for the laboratory. The recommended lower limit of quantification (LOQ) for TAC is ~1 µg/L, as agreed at the 2007 European Consensus Conference on TAC optimization [7].

Controversial results have been derived from clinical studies investigating the concentration–effect relationship for TAC, in contrast to a better defined concentration–toxicity relationship. These discrepant results could be related to limited analytical performance of the assays used in the studies, poor assay standardization and lack of traceability to a single reference material [7–10]. Therefore, recent efforts have focused on assay improvement and standardization. An important step forward was the development of an exact-matching isotope-dilution mass spectrometry method and a certified reference material (ERM-DA110a) by LGC (Teddington, UK) [9,11].

Analysis of TAC in whole blood is performed either by immunoassays or by LC–MS/MS. Results from the Tacrolimus International Proficiency Testing Scheme (IPT) organized by Analytical Services International (ASI) indicate that of the 429 participating laboratories, approximately 60% of the participants use an immunoassay, and 40% use an LC–MS/MS method [12]. LC–MS/MS methods offer favorable analytical specificity and sensitivity with LOQs below 1 µg/L as well as multiplex testing capabilities. However, drawbacks such as instrument costs, lack of automation or 24/24 h technical support, and need for qualified staff render

LC–MS/MS unattractive for many small laboratories [13]. Immunoassays offer around-the-clock results, operational flexibility and relative ease of incorporation into existing automation systems and laboratory workflow, including Laboratory Information System connection [13]. However, reagent costs are relatively high and many assays have limited analytical performance, particularly regarding analytical sensitivity (LOQ between 2 and 4 µg/L) and specificity (cross-reactivity with TAC metabolites) [7]. Immunoassays are susceptible to interferences like cross-reactivity with other drugs and metabolites, reaction with heterophilic antibodies, and influence of endogenous factors like hematocrit or albumin [7,13]. Method imprecision at the lower target therapeutic concentration range is often unsatisfactory, and calibration bias compromises performance. Only two available immunoassays (chemiluminescent microparticle immunoassay (CMIA), Abbott Diagnostics and Quantitative Microsphere System (QMS™), Thermo-Fisher) have a functional sensitivity below 1 µg/L, and were reported to offer adequate accuracy and precision [14–17]. CMIA is developed for the Architect platform. The QMS-based assay can be run on selected open clinical chemistry systems; however, it is very new and more data documenting its analytical performance is needed.

The purpose of the present multicenter evaluation (MCE) study was to evaluate the performance of the new electrochemiluminescence immunoassay (ECLIA) developed by Roche Diagnostics for use on cobas e immunoassay analyzers. Five European laboratories with experience in TDM of immunosuppressive drugs participated in the MCE. Inter-laboratory comparability of the TAC results, agreement with LC–MS/MS (considered reference method in this study), and agreement with the most commonly used commercial immunoassay CMIA were points of particular focus in the MCE.

Table 2
Within-run and intermediate imprecision and bias.

Sample	Target (µg/L)	Site	Instrument	Mean (µg/L)	Within-run imprecision CV (%)	Intermediate imprecision CV (%)	Bias (%)
QC 1	2.5	1	cobas e 411	2.5	4.5	8.1	2.4
	2.6	4	cobas e 602	2.7	3.0	6.9	5.1
QC 2	10.4	1	cobas e 411	10.7	4.0	5.0	2.9
	10.4	4	cobas e 602	10.6	2.4	3.7	1.9
QC 3	19.8	1	cobas e 411	20.6	3.3	5.2	4.0
	19.9	4	cobas e 602	20.1	2.4	3.6	1.0
HSP 1		1	cobas e 411	0.8	8.9	21.0	
HSP 2		4	cobas e 602	1.5	5.1	10.0	
HSP 3		1	cobas e 411	2.5	4.3	8.5	
HSP 4		4	cobas e 602	2.8	3.7	6.5	
HSP 5		4	cobas e 602	4.2	2.9	5.4	
HSP 6		1	cobas e 411	5.5	3.9	6.4	
HSP 7		4	cobas e 602	6.8	2.1	4.4	
HSP 8		1	cobas e 411	9.4	3.1	6.5	
HSP 9		4	cobas e 602	13.0	1.9	3.6	
HSP 10		1	cobas e 411	28.6	2.9	5.9	

The values expressed are the 95% Upper Confidence Limit (UCL).

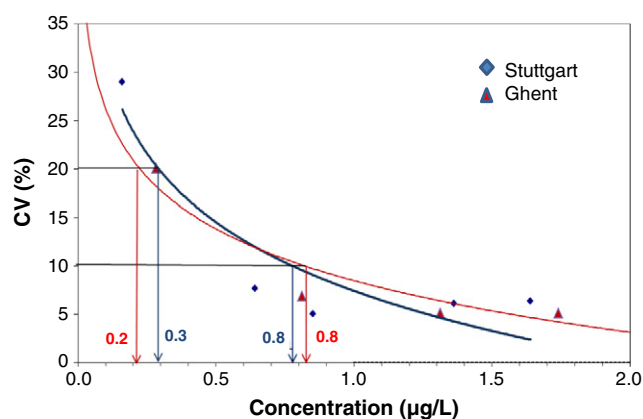


Fig. 1. Functional sensitivity of the ECLIA Tacrolimus assay on the cobas e 411 and cobas e 602 platforms.

Materials and methods

The MCE of the Elecsys Tacrolimus assay was conducted from October 2012 to April 2013 at five European investigational sites: two in Germany (s-1, Klinikum-Stuttgart, Stuttgart, and s-2, Hospital of the University of Munich, Munich), two in Belgium (s-3, Cliniques universitaires St. Luc, Brussels and s-4, Ghent University Hospital, Ghent), and one in Spain (s-5, Hospital Universitari de Bellvitge, Barcelona). Waivers were obtained from the Ethics Committees (EC) of the respective institutions due to use of remnant samples with the exception of Ghent, where samples provided by other sites were used. In addition, all investigational sites conducted the study in accordance with the Declaration of Helsinki (rev. Tokyo, Venice and Hong Kong) and following ICH Good Clinical Practice guidelines.

ECLIA

The Elecsys Tacrolimus immunoassay uses the principle of electrochemiluminescence for detection and measurement [18].

Measurements were performed according to the manufacturer's instructions. The material to be measured (calibrators, quality control (QC) material or patient whole blood specimen) is equilibrated to room temperature (18–25 °C) and mixed gently to resuspend sedimented erythrocytes. Three hundred microliters of material is combined with 0.3 mL of Elecsys ISD sample pretreatment reagent (methanol-based solution containing zinc sulfate) and vortexed for ≥ 10 s. The samples are then centrifuged for 4 min at $\geq 10,000$ g. The supernatant is decanted into a Hitachi sample cup and capped until loading onto the system. As recommended by the manufacturer no batches of more than 30 samples were processed. Sample preparation time for one batch of 30 samples is approximately 20 to 30 min, and time to first result is 18 min. In three institutions (s-1, s-2 and s-3) the cobas e 411 platform was used for the evaluation, whereas in s-4 a cobas e 602 analyzer and in s-5 a cobas e 601 analyzer were used.

The Elecsys Tacrolimus assay is calibrated using the Elecsys Tacrolimus Calset with two concentration levels. Calibrators were reconstituted according to the manufacturer's instructions and stored in 0.3 mL aliquots for 7 days at 2–8 °C or below -15 °C for 28 days. After sample pretreatment they were used for calibration within 30 min of preparation. Calibration was performed once per reagent lot, and as required for maintaining quality control values within specified limits. The lot calibration stability period is 28 days. The Elecsys Tacrolimus Calset traceability to USP material recovery is in close agreement with the certified reference material ERM DA110a. According to the manufacturer's data, ERM DA110a measurements on four cobas e 411 and two cobas e 601 analyzers at the manufacturer's site using two different lots of the Elecsys Tacrolimus reagent yielded a bias $<5\%$ compared to the certified value. The intermediate imprecision of ERM DA110a was $<5\%$ (Supplemental Data Table 1).

The QC material, PreciControl ISD (PC ISD) at three concentration levels, was provided by Roche Diagnostics for use on the cobas analyzers. The QC samples were prepared and stored similarly to the calibrators. Each instrument run was validated by measuring the QC material before patient sample material was investigated.

ECLIA assay cross-reactivity as reported by the manufacturer is 70% for metabolite M II and is not detectable for M I, M III, and M IV. No impact on assay results was observed from interference by bilirubin at

Table 3

Mean bias between method specific TAC concentrations found with the EQAS samples in the MCE and the CMIA overall mean results from the EQAS group as compared to the EQAS group LC-MS^a means.

IPT sample number	194		195		201	203	204	205	206	208
	A spiked	A pooled patient	B spiked	C spiked	B spiked	B spiked	A spiked	C spiked	C pooled patient	A pooled patient
HPLC-MS overall										
Mean \pm SD (μ g/L)	15.1 \pm 1.2	10.6 \pm 0.8	5.1 \pm 0.5	19.1 \pm 1.5	14.5 \pm 1.3	6.6 \pm 0.6	2.1 \pm 0.3	15.4 \pm 1.4	6.0 \pm 0.5	7.0 \pm 0.5
(N)	(138)	(144)	(144)	(144)	(148)	(157)	(152)	(155)	(156)	(159)
ECLIA, MCE										
Mean \pm SD (μ g/L)	16.2 \pm 1.0	11.8 \pm 0.5	5.3 \pm 0.3	20.1 \pm 1.5	15.9 \pm 0.9	7.1 \pm 0.3	2.0 \pm 0.1	16.4 \pm 0.4	6.5 \pm 0.5	7.4 \pm 0.3
Bias ^b (%)	7.3	11.3	3.9	5.2	9.7	7.6	-4.8	6.5	8.3	5.7
(N)	(12)	(12)	(12)	(12)	(15)	(15)	(15)	(6)	(15)	(6)
LC-MS/MS, MCE										
Mean \pm SD (μ g/L)	15.7 \pm 2.0	10.6 \pm 0.5	4.8 \pm 0.3	20.2 \pm 2.5	14.9 \pm 1.8	6.6 \pm 0.3	2.1 \pm 0.1	16.5 \pm 1.4	6.1 \pm 1.0	7.2 \pm 0.9
Bias ^b (%)	4.0	0.0	-5.9	5.8	2.8	0.0	0.0	7.1	1.7	2.9
(N)	(9)	(9)	(9)	(9)	(15)	(15)	(15)	(6)	(15)	(6)
CMIA overall ^c										
Mean \pm SD (μ g/L)	15.8 \pm 1.0	11.5 \pm 0.7	5.2 \pm 0.3	19.8 \pm 1.2	15.1 \pm 1.2	6.9 \pm 0.5	2.0 \pm 0.2	16.5 \pm 1.0	6.5 \pm 0.4	7.6 \pm 0.4
Bias ^b (%)	4.6	8.5	2.0	3.7	4.1	4.5	-4.8	7.1	8.3	8.6
(N)	(151)	(151)	(151)	(151)	(155)	(156)	(144)	(148)	(149)	(154)
CMIA, MCE										
Mean \pm SD (μ g/L)	14.9 \pm 1.1	11.5 \pm 0.7	5.1 \pm 0.4	19.0 \pm 1.0	15.8 \pm 1.2	7.3 \pm 0.7	2.1 \pm 0.2	16.8 \pm 0.9	6.3 \pm 0.7	8.5 \pm 0.2
Bias ^b (%)	-1.3	8.5	0.0	-0.5	9.0	10.6	0.0	9.1	5.0	21.4
(N)	(6)	(6)	(6)	(6)	(9)	(9)	(9)	(3)	(9)	(3)

n.m. = not measured.

^a This IPT group combined LC-MS and LC-MS/MS method results.

^b "Bias" is measured against the HPLC-MS overall results from the EQAS group.

^c CMIA in the IPT reports is defined as "CMIA – on Architect platform" or "Architect".

$\leq 1026 \mu\text{mol/L}$, from albumin at $\leq 12.0 \text{ g/dL}$, and from hematocrit in the range of 15–60% [19].

TAC comparator analytical systems and LC–MS/MS

According to the study protocol experiments on comparator systems Abbott Architect and LC–MS/MS were included. The Architect platform was utilized in s-1, s-3 and s-4, and LC–MS/MS at all participating sites. Comparison to Siemens Dimension was specified in the protocol but experiments were discontinued due to manufacturer reagent shortages.

Sample pretreatment for the CMIA and measurement on the Abbott Architect was performed according to the manufacturer's instructions and according to site-specific standard operating procedures. Calibration was performed using assay specific materials provided by the vendor, which were not traceable to ERM-DA110a at the time of the study.

Each investigational site performed LC–MS/MS according to the protocols developed and routinely used at the site (Table 1). The calibrator set provided by Chromsystems was traceable to ERM-DA110a in contrast to the set from Recipe at the time of this evaluation.

Each participating laboratory has a routine internal QC program that was followed for the measurements on the comparator systems, as well as successful regular participation in external quality assessment schemes (EQAS).

Sample sources and handling

EDTA whole blood samples from patients who had received a heart (HTx), kidney (KTx) or liver (LTx) transplant, under TAC therapy (more than 60 per cohort), were collected at the evaluation sites or at the Institute for Clinical Chemistry, Medical University of Hannover under the same IRB/EC and GCP guidelines as outlined above. Commercial EQAS samples were purchased from ASI, UK.

Samples not collected at site were shipped frozen on dry ice and stored at $\leq -15^\circ\text{C}$ until analysis. Samples collected at site were stored at room temperature ($18\text{--}25^\circ\text{C}$) if tested within 8 h of collection, or at $2\text{--}8^\circ\text{C}$ if tested within one week. If longer storage was necessary, samples were stored below -15°C . Samples were measured within 6 months after collection. Liquid whole blood aliquots used for the method comparison were measured within 24 h using all methods and stored at $2\text{--}8^\circ\text{C}$. Aliquoted samples did not undergo more than one freeze/thaw cycle.

Discrepant results

Discrepant results were compared to the respective result obtained with LC–MS/MS, and all samples with greater than 40% difference to LC–MS/MS results were treated as outliers. Testing was repeated in triplicate on the analyzer which showed the discrepant value when compared to LC–MS/MS.

Imprecision and accuracy

Assay imprecision was tested in s-1 (cobas e 411) and s-4 (cobas e 602) according to protocol CLSI EP5-A2 [20]. Imprecision was determined using 84 aliquots each of PC ISD material at 3 concentration levels and 5 pooled EDTA whole blood samples with concentration between 0.5 and $40 \mu\text{g/L}$ (Table 2). Measurements reflect a combination of pre-analytical and instrumental variations. Controls and samples were stored frozen in 0.3 mL aliquots and treated for analysis as described above. One run was performed per day over 21 days. Samples were randomized and measured in duplicate. Accuracy was calculated using the measurement results from the PreciControl ISD material. Lab-to-lab precision was calculated according to Searle et al. [21] with the three levels of ISD QC materials and the EQAS samples (spiked $n = 3$; pooled patient $n = 1$) analyzed at all five sites.

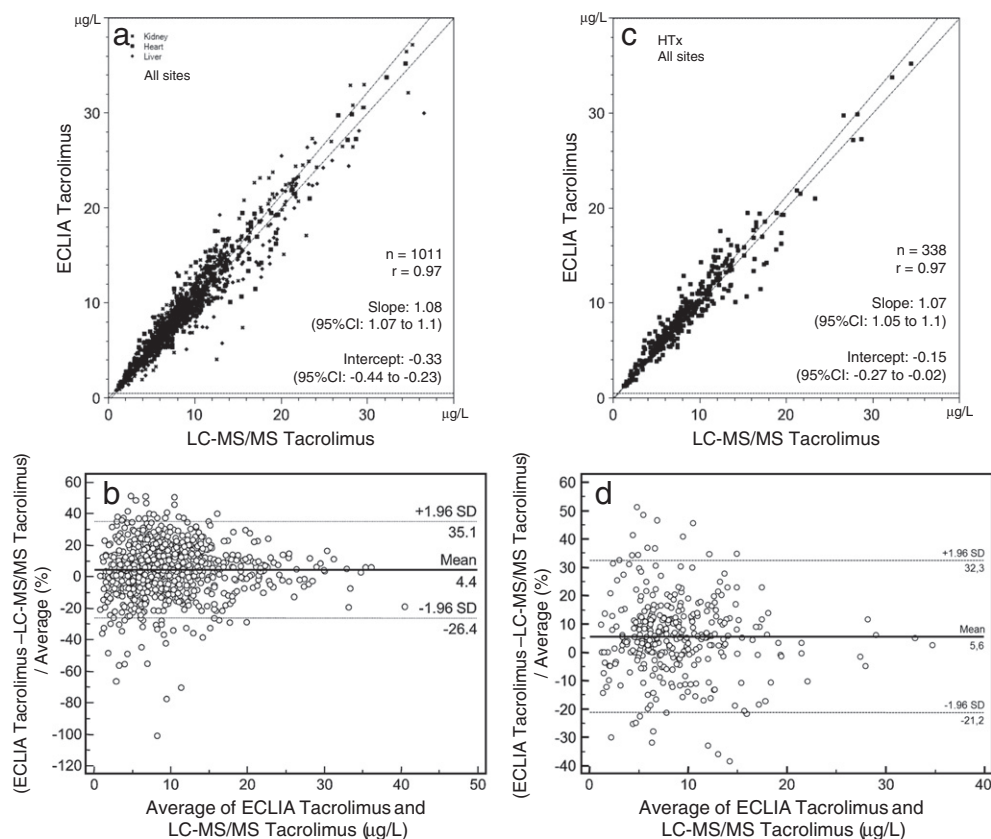


Fig. 2. a–h. Method comparison of TAC assays with Deming regression and Bland–Altman analysis of LC–MS/MS versus ECLIA for all Tx types (a) and (b), HTx (c) and (d), KTx (e) and (f), and LTx (g) and (h).

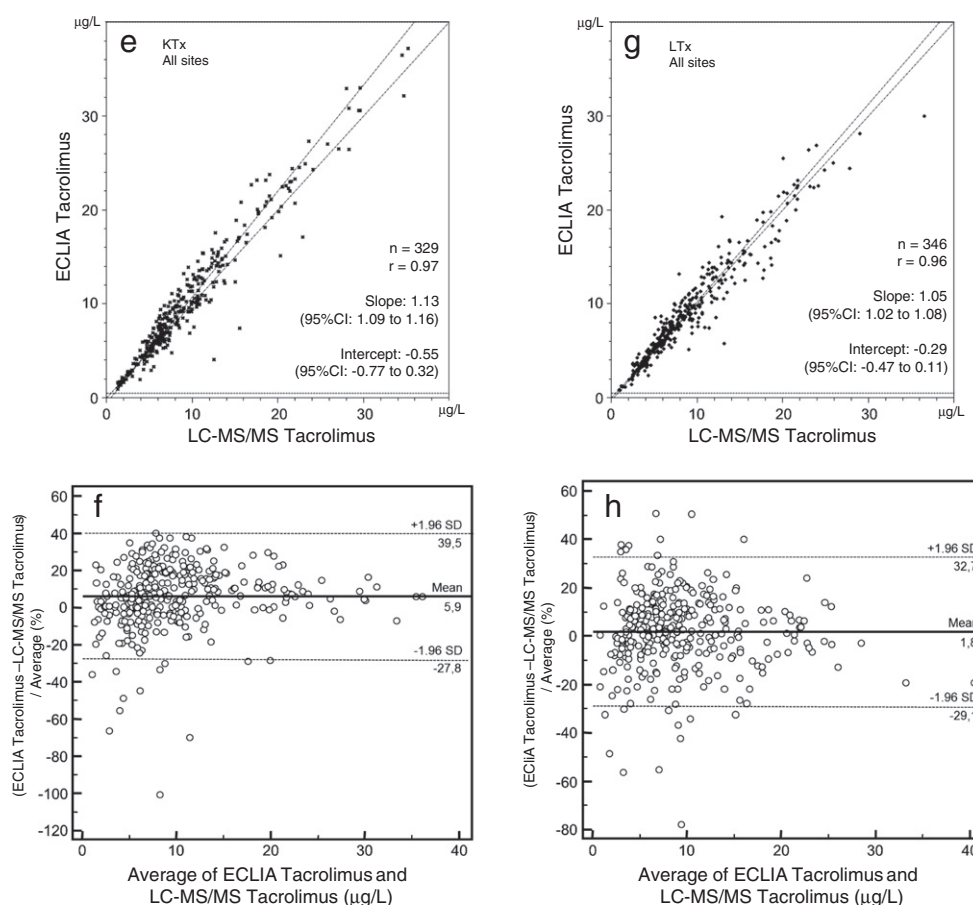


Fig. 2 (continued).

Linearity

Linearity experiments were performed in s-1 (cobas e 411) and s-4 (cobas e 602) according to the polynomial method described in CLSI EP6-A [22]. As the medically relevant range for trough sampling covers the lower end of the measuring range [$<15 \mu\text{g/L}$], a quadratic weighting function was applied to the test results of the dilution series. Two pools (a native and a spiked high concentration pool) were used to prepare the 15-step dilution series. Three replicates per dilution step were measured in 1 run. Regression analysis for first-, second- and third-order polynomials was calculated.

Functional sensitivity

Functional sensitivity was assessed in s-1 (cobas e 411) and s-4 (cobas e 602). Pooled EDTA whole blood samples with target concentrations at approximately 0.3, 0.9, 1.2, 1.6 and $2.0 \mu\text{g/L}$ were used and 14 aliquots with a volume greater than 0.3 mL were prepared. Samples were measured in 1 run each day for 10 days in total with a single measurement per aliquot.

Determination of TAC in EQAS samples

Selected EQAS samples from the IPT organized by ASI were measured at all investigational sites on both cobas and LC-MS/MS instruments and on the Architect system in the sites performing the CMIA. All instruments were calibrated before the experimental run. Samples were divided into 0.3 mL aliquots and measured in 1 run with 3 replicates for each sample. For each EQAS sample, bias relative to the mean LC-MS/MS value from the overall EQAS group results was calculated for the CMIA, ECLIA, and LC-MS/MS (MCE) method means.

Method comparison

Anonymized residual samples were measured with one replicate per method. Comparisons were calculated using weighted Deming regression. Results were rated against the following criteria (Oellerich et al.) as a guideline [23]: slope 1.0 ± 0.10 , intercept $<1/10$ of the low end of the therapeutic concentration range ($3.0 \mu\text{g/L}$ for KTx and $5 \mu\text{g/L}$ for HTx and LTx during the stable clinical phase after transplantation [7]), and Pearson's correlation $r \geq 0.97$. To gain further information on method comparability, Bland-Altman difference plots [24] were evaluated.

Statistical analysis

Statistical analysis was performed using the Windows based Computer Aided Evaluation (WinCAEv) program [25] except for the Bland-Altman difference plots which were generated using the MedCalc software (Oostende, Belgium). Parametrical characteristics such as mean, SD, and Pearson's r were used after confirmation that data being analyzed are normally distributed.

Results

Linearity

Elecsys Tacrolimus was shown to be linear from $0.5 \mu\text{g/L}$ to $40 \mu\text{g/L}$. The maximum difference between linear and quadratic models was 13%, the maximum difference between linear and cubic models was 14.8%. The absolute deviation increases towards the end of the measuring range as expected based on the weighting function applied to the dilution series results (Supplemental Data Fig. 1).

Functional sensitivity

Plotting the CVs determined from 10 single measurements over 10 days at each concentration against the mean concentration (Fig. 1) demonstrates a 20% CV for a TAC concentration of 0.2 µg/L in s-4 and 0.3 µg/L in s-1. A 10% CV was related to 0.8 µg/L TAC at both sites. The bias of the measurement at the single concentration points ranged between 1.2% and 4.4% in s-1 and between 0.5% and 3.3% in s-4. From these data the functional sensitivity (CV ≤ 20%) was determined to be 0.3 µg/L.

Assay imprecision

The within-run and intermediate imprecision determined in s-1 and s-4 are presented in Table 2. The within-run imprecision was ≤ 5.1% over the concentration range investigated on cobas e 602 (5.1% at 1.5 µg/L) and ≤ 8.9% (8.9% at 0.8 µg/L) on cobas e 411. The respective intermediate imprecision was ≤ 10.0% in s-4 (10.0% at 1.5 µg/L) and ≤ 21.0% in s-1 (21.0% at 0.8 µg/L).

The lab-to-lab imprecision found with the three levels of the ISD QC materials ranged between 8.5% (QC3, mean TAC concentration 19.1 µg/L) and 12.1% (QC1, mean TAC concentration 2.3 µg/L). The EQAS samples (spiked n = 3; pooled patient n = 1) analyzed at all five sites yielded a lab-to-lab imprecision between 4.7% (spiked sample with mean TAC concentration of 7.1 µg/L) and 7.3% (pooled patient sample with mean TAC concentration of 6.5 µg/L).

Assay inaccuracy

ECLIA assay inaccuracy was evaluated with 3 levels of quality control samples provided by Roche (ISD) in s-1 and s-4 and with 6 to 8 EQAS

samples, including 3 pooled patient whole blood samples at all investigational sites (Tables 2 and 3). The number of EQAS samples analyzed at the five sites was different due to partly insufficient sample availability. Using the ISD materials a bias of ≤ 4.0% was found on cobas e 411 and ≤ 5.1% on cobas e 602. The mean bias found using ECLIA with spiked EQAS samples was ≤ 9.7%, whereas with pooled patient samples it was ≤ 11.3%. The EQAS samples with the highest site specific deviations from target were not the same at the different sites. There was no apparent relationship between TAC concentrations and the bias found (data not shown).

Evaluation of assay inaccuracy was extended by including measurements of the same EQAS samples with both comparison methods (LC-MS/MS and CMIA, Table 3). Analysis of all samples at all sites was not possible due to insufficient sample availability. With LC-MS/MS a mean bias of ≤ 7.1% with the spiked EQAS samples and ≤ 2.9% with the pooled patient EQAS samples were found. The respective results for the EQAS samples with CMIA were ≤ 10.6% and ≤ 21.4%. As noted before for ECLIA, the EQAS samples with the highest site specific deviations from the targets were not identical at the different sites and a systematic bias was not observed (data not shown).

Method comparison

Comparability of ECLIA results to LC-MS/MS (Fig. 2) and CMIA (Fig. 3) was separately investigated for specimens derived from KTx, HTx, and LTx recipients using Deming regression analysis. No systematic between-site deviation of EQAS sample results for a given assay was observed (data not shown). Therefore, assay-specific results were combined for the evaluation. Comparing LC-MS/MS to ECLIA and using the criteria of Oellerich et al. [23], values outside the acceptance were found only for slope (1.13 (95%CI: 1.09/1.16) vs. 1.0 ± 0.1) with KTx patient specimens. The respective slope values for all Tx, HTx and LTx

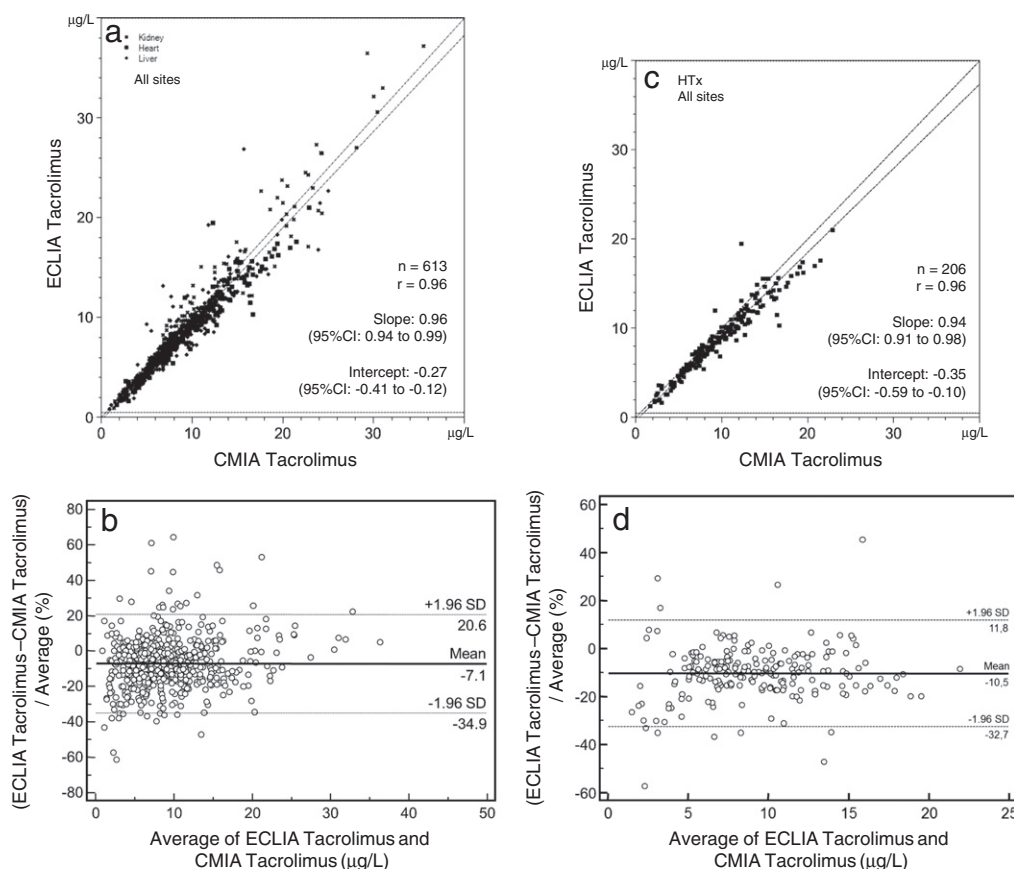


Fig. 3. a–h. Method comparison of TAC assays with Deming regression and Bland–Altman analysis of CMIA versus ECLIA for all Tx types (a) and (b), HTx (c) and (d), KTx (e) and (f), and LTx (g) and (h).

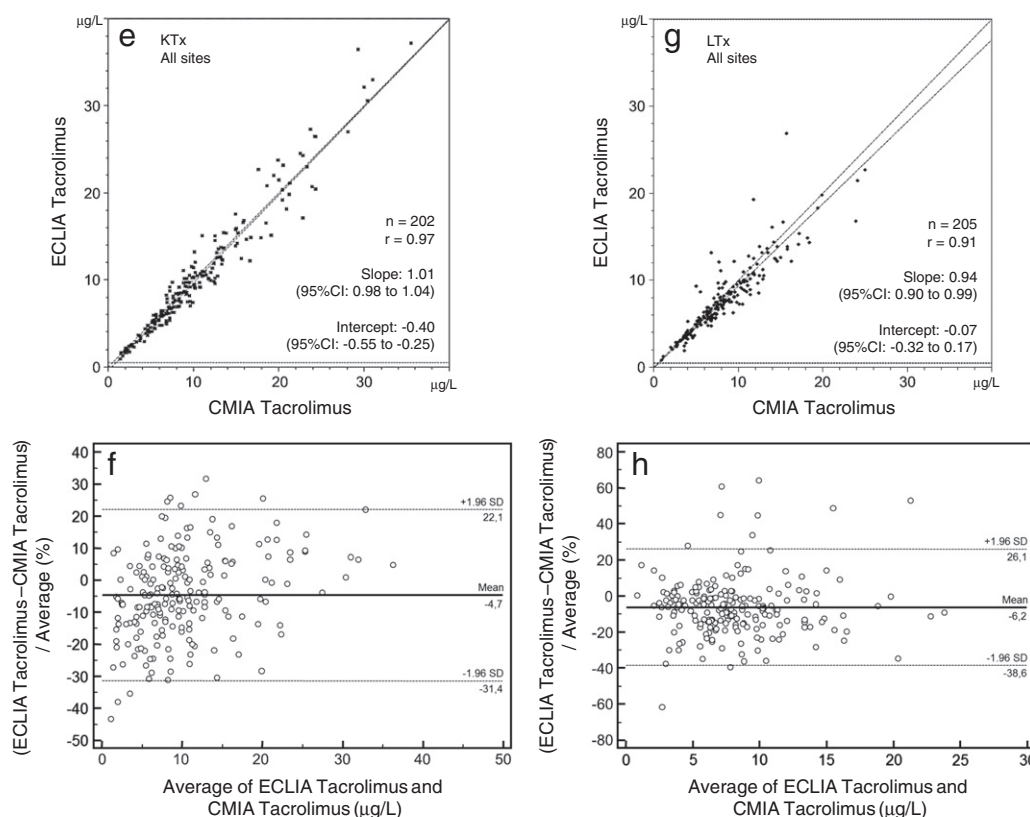


Fig. 3 (continued).

patients were 1.08 (95%CI: 1.07/1.10), 1.07 (95%CI: 1.05/1.10) and 1.05 (95%CI: 1.02/1.08). Pearson's correlation r was very similar for the global comparison and the three patient groups (all: 0.97, heart: 0.97, kidney: 0.97 and liver: 0.96). Bland–Altman difference plots for ECLIA vs. LC–MS/MS (Fig. 2b,d,f,h) revealed a combined average bias of 4.4% over the five sites (± 1.96 SD: -26.4 – 35.1%) for all Tx, 5.6% (± 1.96 SD: -21.1 – 32.3%) for HTx, 5.9% (± 1.96 SD: -27.8 – 39.5%) for KTx, and 1.8% (± 1.96 SD: -29.1 – 32.7%) for LTx.

Site-specific analysis of method comparison between ECLIA and LC–MS/MS revealed more significantly divergent results (Supplemental Data Table 2). Whereas the acceptance criteria mentioned above were met for all patient groups in s-1, for KTx and LTx in s-5 (exception: slope 1.20 with HTx, 95%CI: 1.13/1.28) as well as for HTx and LTx in s-2 (exception: slope 1.19 with KTx, 95%CI: 1.11/1.28), multiple deviations were found in s-3 and s-4. In s-3 the calculated slopes were consistently above 1.10 (HTx 1.20 with 95%CI: 1.15/1.26, KTx 1.32 with 95%CI: 1.25/1.38, and LTx 1.16 with 95%CI: 1.05/1.28). In s-4 the Pearson's r was always < 0.97 (HTx 0.94, KTx 0.94, LTx 0.88). A site specific evaluation using the Bland–Altman difference plot analysis revealed a range of bias means between -5.5% (s-4) and 15.6% (s-5) for HTx, between -11.1% (s-4) and 23.3% (s-3) for KTx, and between -9.3% (s-4) and 10.3% (s-3) for LTx.

Comparing ECLIA data to CMIA, an intercept between -0.40 μg/L (KTx, 95%CI: -0.552 to -0.249) and -0.07 μg/L (LTx, 95%CI: $-0.318/0.172$) was observed (Fig. 3). The slope was 0.96 for all Tx, 0.94 for HTx and LTx and 1.01 for KTx. Pearson's correlation r ranged from 0.91 (LTx) to 0.97 (KTx). The respective Bland–Altman difference plots (Fig. 3b,d,f,h) displayed a combined average bias of -7.1% (± 1.96 SD: -34.9 – 20.6%) for all Tx, -10.5% (± 1.96 SD: -32.7 – 11.8%) for HTx, -4.7% (± 1.96 SD: -31.4 – 22.1%) for KTx and -6.2% (± 1.96 SD: -38.6 – 26.1%) for LTx.

Discrepant results were observed for 72 samples (4%). After re-analysis, the discrepancy was resolved in 61 cases and remained in 11 cases.

Discussion

In this study the analytical performance of the new Elecsys Tacrolimus assay was evaluated in five European laboratories experienced in routine TAC concentration monitoring.

Results of the experiments performed at s-1 and s-4 confirmed method linearity up to 40 μg/L and LOQ of 1 μg/L, as stated in the package insert [19]. Thus the ECLIA performance demonstrated in this MCE fulfilled the recommendations of the 2007 European Consensus Conference. The upper limit of quantification is higher than limits reported with other immunoassays currently in use but lower than limits reported with the LC–MS/MS technique [17,26,27]. A high upper limit of quantification may be advantageous for the evaluation of TDM strategies including TAC concentrations close to the peak [7,28–30].

According to international recommendations [23] an imprecision below 5% at middle and high therapeutic concentrations and better than 10% for concentrations at the lower end of the therapeutic range should be achieved with assays for immunosuppressive drugs. Within-run imprecision $< 5\%$ over the range 2.47 – 28.6 μg/L was found both in s-1 and s-4. The intermediate imprecision for TAC concentrations ≥ 6.8 μg/L was $< 5\%$ in s-4, whereas in s-1 the CVs ranged between 5.0 and 6.5%. At lower therapeutic concentrations (to 1.5 μg/L) it was consistently $\leq 10\%$ (Table 2). Data generated with 4 EQAS samples in the five sites demonstrated a lab-to-lab imprecision of $\leq 7.3\%$. These imprecision results are well comparable to those reported with the CMIA and LC–MS/MS and favorable when compared to previously developed immunoassays [7,17,26,27]. The mean bias to LC–MS/MS (EQAS group means) found using ECLIA with spiked EQAS samples was $\leq 9.7\%$ and with pooled patient samples $\leq 11.3\%$. This accuracy was confirmed by TAC concentrations measured with ECLIA in transplant patients' whole blood samples compared to LC–MS/MS results. Combined data from the five sites analyzed by Deming regression and Pearson's correlation revealed values within the acceptance criteria [23] except for KTx samples which violated the slope. Since the slope with KTx samples was

significantly above 1.0 at all sites except s-4 (1.12, but $p > 0.05$) deviation may result from cross-reactivity, calibration bias or instrument settings. The same trend of proportional error (slope > 1.0) was also observed for HTx and LTx samples throughout the sites. However, the average deviation from the acceptance criteria was smaller and criteria were met.

Between-site differences were observed when evaluating local results from single patient groups (Supplemental Data Table 2), suggesting that site-specific factors may be important. Whereas the lots of ECLIA reagent, calibrators and QC materials were identical at all sites, the LC-MS/MS methods were developed “in-house” with different sample pretreatment and assay conditions (Table 1). ECLIA was calibrated three to six times per site during the method comparison, whereas for LC-MS/MS a daily calibration is usual. The ECLIA uses a 2-point calibration, but a 7-point calibration is most commonly applied for LC-MS/MS. In addition, the patient samples used for the method comparisons were not identical at all sites.

Cross-reactivity from TAC metabolites with ECLIA was reported only for M-II (31-O-Demethyl-TAC, ~70%) [19]. M-II has comparable pharmacological activity as TAC itself and reaches about 15% relative to TAC steady state concentrations. This cross-reactivity is lower than that demonstrated for the CMIA (94%) but higher compared to the antibody-conjugated magnetic immunoassay (ACMIA, 2.7%) [17,31]. It can explain a bias up to 11% between the ECLIA and LC-MS/MS in human samples that is in agreement with the results found in this study. Whereas cross-reactivity of pharmacologically inactive TAC metabolites was also reported for CMIA (~45%) and ACMIA (~18%), ECLIA is not affected [17,31]. Considering these differences in cross-reactivity and the ratio of cross-reacting metabolites to TAC in steady state, it can be expected that the bias between these immunoassays associated with limited antibody specificity will not exceed 6–7%. In fact, this assumption is supported by the results of the method comparisons between ECLIA and CMIA.

Between-method bias is important for establishing therapeutic ranges when using different assays within one lab, between labs or when replacing a method. Bland–Altman analysis demonstrated an average bias of $< +6\%$ between ECLIA and LC-MS/MS with ≥ 300 samples each from HTx, KTx and LTx patients (corresponding to 0.4 $\mu\text{g/L}$ with HTx, 0.7 $\mu\text{g/L}$ with KTx, and 0.1 $\mu\text{g/L}$ with LTx on average). The average bias of ECLIA against CMIA was $\leq +10.5\%$ (corresponding to 0.9 $\mu\text{g/L}$ with HTx, 0.2 $\mu\text{g/L}$ with KTx, and 0.5 $\mu\text{g/L}$ with LTx). Considering the analytical performance of these methods no modification of therapeutic ranges for TAC is required when switching between these methods. However, at some sites larger differences were found according to Bland–Altman analysis (e.g. $+15.6\%$ at s-5 with HTx samples and $+23.3\%$ at s-3 with KTx samples vs. the local LC-MS/MS method). Therefore, if laboratories use locally established therapeutic ranges, they should be cautious with transferring these ranges between methods.

In conclusion, the over-all performance of ECLIA in this MCE demonstrates a modern generation TAC assay that meets the demands of monitoring drug concentrations in current immunosuppressive regimens. The assay performed well at all investigator sites, implementation and handling were simple, no deviation from the manufacturer insert information regarding calibration and/or reagent stability was observed. Regarding assay handling under routine conditions, it should be noted that 300 μL of sample is needed which may become limiting with pediatric samples. In addition, the use of open secondary cups with a methanol-based precipitation solution restricts the length of the batches due to the risk of a positive bias due to evaporation. In this study, identical lots of calibrator, reagents and QC materials were used in all laboratories, necessitating further independent studies to evaluate over-all method stability. Finally, in addition to the ECLIA evaluation the experience generated with different TAC assays in this MCE clearly supports the importance of method standardization to ensure comparable results and high quality of patient care.

Conflict of interest

MS has received fees from Siemens for scientific presentations. MV has received fees from Roche for scientific presentations. AV has received fees from Roche Diagnostics Belgium for scientific presentations in 2012. CS is an employee of Roche Diagnostics Germany. PW has received fees from Roche for scientific presentation on immunosuppressive drugs TDM. For the remaining authors none were declared. This study was sponsored by Roche Diagnostics Germany GmbH.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clinbiochem.2014.03.023>.

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